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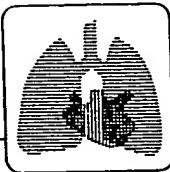
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clinical implications of basic research

Fibronectin*

A Versatile Matrix Protein with Roles in Thoracic Development, Repair and Infection

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Fibronectin, a dimeric cell-adhesive extracellular matrix glycoprotein, is secreted by mesenchymal cells and assembled into insoluble matrices which have important biological functions in embryologic development as well as in tissue response to injury. Fibronectin interacts with numerous cell types including mesenchymal cells and inflammatory cells which bear appropriate fibronectin receptors. *In vitro*, fibronectin serves as an adhesive substrate and promotes cell proliferation and cytodifferentiation. During development, fibronectin-rich matrices are deposited in specific location and regulate the directional migration of embryonic cells. In particular, fibronectin matrices appear to be of critical importance to normal cardiopulmonary development. Following embryologic development, the tissue expression of fibronectin is greatly reduced, but increases markedly following tissue injury, where newly expressed

fibronectin matrices appear critical to tissue repair. Recent evidence has documented increased expression of fibronectin in numerous pulmonary conditions including the adult respiratory distress syndrome (ARDS), bronchiolitis obliterans organizing pneumonia (BOOP) and idiopathic pulmonary fibrosis (IPF). Additionally, fibronectin also interacts with a large number of microorganisms and therefore also is potentially important in microbial adherence to airway epithelium and subsequent infections of the respiratory system. (Chest 1992; 101:1663-73)

AIP = acute interstitial pneumonitis; BOOP = bronchiolitis obliterans organizing pneumonia; cAMP = cyclic adenosine 5'-monophosphate; IPF = idiopathic pulmonary fibrosis; mRNA = messenger ribonucleic acid; TGF- β = transforming growth factor β ; VLAs = very late activation antigens

Fibronectins are remarkably versatile cell adhesive glycoproteins capable of interacting with macromolecules including fibrin, collagen, proteoglycans as well as cells bearing specific fibronectin receptors on their surfaces.¹ Fibroblasts and other mesenchymal cells synthesize, secrete and bind soluble dimeric fibronectin and catalyze its conversion to insoluble multimeric disulfide-cross-linked extracellular matrices.² In turn, fibronectin-rich extracellular matrices have multiple biologic functions in embryogenesis and tissue repair including cell attachment, migration, proliferation and cytodifferentiation.³

The deposition of fibronectin matrices at specific locations during embryogenesis is critical for the migration of cells and subsequent organ morphogen-

esis.⁴ In contrast to the embryonic period, the expression of fibronectin is quite restricted after birth, limited primarily to hepatocytes which secrete soluble fibronectin into the circulation where it represents 30 mg percent of the circulating blood protein.¹ Additionally, normal alveolar macrophages constitutively secrete fibronectin into the alveolar space where it can be detected at a concentration of 7 to 8 μ g/mg of albumin in BAL of normal volunteers.⁵

Following tissue injury, the expression of fibronectin increases dramatically. Enhanced fibronectin expression is noted in a variety of lung disorders including ARDS, BOOP and IPF.^{6,7} Enhancement of fibronectin expression in these disorders is noted both in alveolar macrophages and resident fibroblasts derived from the pulmonary interstitium. Fibronectin is increased roughly 50 percent in the BAL of smokers.⁸ Exposure to a number of other injurious agents including cobalt chloride, nickel and asbestos also causes alveolar macrophages to increase their production of fibronectin.⁹ Fibronectin may serve multiple functions in tissue repair and fibrosis acting as a chemoattractant and adhesive substrate for mesenchymal and epithelial cells which migrate into damaged tissues.

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This work was supported in part by funds from the American Heart Association (Clinician-Scientist Award No. 91004230 to A.H.L.) and by a Minority Medical Faculty Development Award from The Robert Wood Johnson Foundation (to Dr. Roman).

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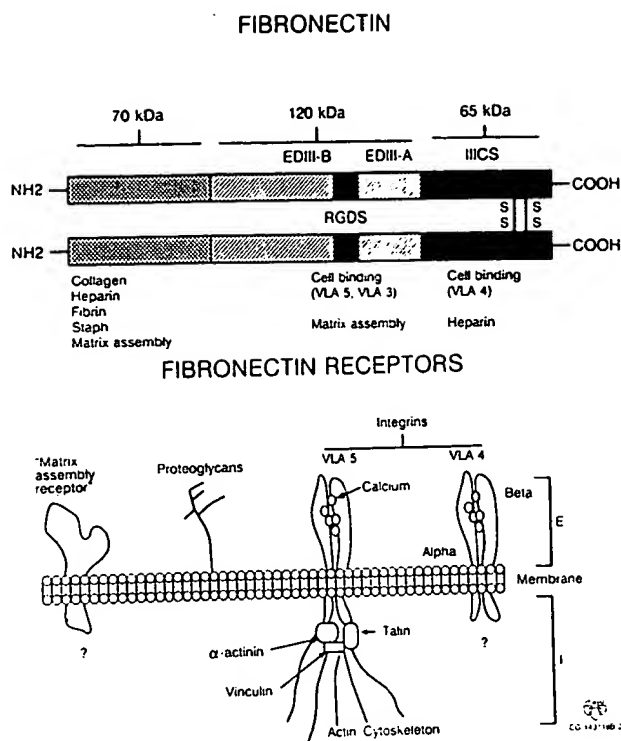


FIGURE 1. *Top*: Structure of fibronectin. Fibronectin is a dimeric glycoprotein that contains several functional domains. Enzymatic digestion of the fibronectin molecule results in fragments that retain their biologic activity after purification. *Bottom*: Structure of fibronectin receptors. The fibronectin molecule is bound by multiple cell surface components which include the putative matrix assembly receptor, proteoglycans and integrins. *E* denotes extracellular and *I* denotes intracellular regions of the molecules.

In addition to interacting with macromolecules and receptor armed cells, fibronectins also interact with a large number of microorganisms including bacteria, fungi and protozoans.¹⁰ Potential functions of these fibronectin-microbe interactions are still being elucidated but potentially include enhanced adherence of organisms to host epithelial and phagocytic cells. In view of fibronectin's varied and biologically important interactions with blood and matrix proteins, cells and microorganisms, it is of little surprise that this versatile molecule has been the center of intense biochemical and cellular research and has achieved a central role in the study of cardiopulmonary biology.

FIBRONECTIN BIOCHEMISTRY

Fibronectins are dimeric glycoproteins of approximately 500 kDa molecular weight which are derived from the variable splicing of a single gene product. Fibronectins are strikingly modular proteins composed of protease-resistant domains which retain their individual activities following cleavage and purification from the intact molecule (Fig 1, *top*). These protease-resistant domains are in turn composed of smaller homologous motifs termed type I, II and III repeats which comprise the majority of fibronectin's protein structure.¹ At least 20 different forms of fibronectin

have been described, resulting from the variable splicing of a single gene product located on chromosome 2 in man.¹¹ Three regions of fibronectin are variably spliced; the EDIIA, EDIIB and the HIICS regions. The EDIIA region is present on fibronectin derived from fibroblasts and other mesenchymal cells (cellular fibronectin) but is absent from fibronectin secreted by hepatocytes (plasma fibronectin).¹¹ The function of the EDIIB region is currently unknown. The variably spliced HIICS region is present on some forms of fibronectin and appears to be important in mediating fibronectin binding to lymphocytes.¹² All forms of fibronectin are glycosylated through both N- and O-linked modification, with carbohydrate comprising 5 percent of the apparent molecular weight.¹³

The ability to cleave and purify the domains of fibronectin has permitted biochemists to dissect the varied biologic functions of fibronectin and map these activities to distinct regions of the molecule (Fig 1, *top*). The aminoterminal 70 kDa domain of fibronectin provides fibronectin with ability to bind noncovalently to other extracellular matrix components including collagens and proteoglycans.¹ This portion of the molecule additionally binds covalently to fibrin present in blood clots through a transglutaminase-mediated reaction catalyzed by coagulation factor XIIIa. The most aminoterminal 29 kDa region of the molecule (contained within the 70 kDa domain) is essential for the assembly of soluble fibronectin into insoluble disulfide crosslinked extracellular matrices.¹⁴ The central 120 kDa domain of fibronectin contains the Arg-Gly-Asp-Ser (RGDS) adhesive sequence, which binds to the classic integrin fibronectin receptor, VLA-5, expressed on mesenchymal cells such as fibroblasts, monocytes and alveolar macrophages, as mentioned by Ruoslahti¹ and as noted by A. H. Limper, M.D., T. J. Broekelman, M.D., and J. A. McDonald, M.D. (unpublished observations). The carboxyterminal 65 kDa domain of fibronectin contains the HIICS or variable region which binds to another fibronectin receptor termed VLA-4, present on lymphocytes.¹² The carboxyterminal domain of one fibronectin chain is covalently cross-linked to a similar chain to form the soluble dimeric fibronectin molecule. Only dimeric forms of fibronectin can be successfully incorporated into the extracellular matrix.²

A variety of inflammatory cytokines have been implicated in regulating fibronectin gene expression. Platelet-derived growth factor, vitamin D₃, interleukin-6, serum and TGF- β cause fibroblasts to increase their secretion of fibronectin *in vitro*.¹⁵ The effects of cytokines on fibronectin expression are mediated primarily by enhanced synthesis of fibronectin mRNA. However, cAMP increases fibronectin expression by lengthening fibronectin mRNA half-life. The cytokine TGF- β is of particular importance in controlling the

local abundance of fibronectin and dramatically increases the gene expression of both fibronectin and the VLA-5 integrin fibronectin receptor.¹⁶ In addition, TGF- β also suppresses the secretion of fibroblast-derived proteases, thus further augmenting the net deposition of fibronectin-rich matrices.

FIBRONECTIN RECEPTORS

Cells interact with fibronectin via specific cell surface receptors (Fig 1, *bottom*).¹ Fibronectin receptors include members of the integrin family, a structurally related group of transmembrane heterodimeric glycoproteins conserved during evolution and responsible for cell binding to extracellular matrices or to other cells.¹⁷ Integrin receptors are composed of distinct α subunits that help confer ligand binding specificity and β subunits common to other integrins. Traditionally, integrins have been classified into subfamilies depending on their β subunit (β 1, 2, 3, ...).^{18,19} However, recent evidence has shown that some α subunits may bind with multiple β subunits, making such classification schemes misleading.

Integrins containing β_1 subunits include receptors for fibronectin, collagen and laminin. The β_2 -containing integrins, also termed leucams, are found exclusively in leukocytes and include receptors that mediate cell-cell interactions critical for the immune response. Integrins containing β_3 subunits have been termed cytoadhesins and include receptors for vitronectin, fibrinogen, von Willebrand factor and thrombospondin and are found in platelets, endothelial cells and certain cancer cells. New integrins currently are being identified and their functions are under study.

Integrin subunits contain prominent aminoterminal extracellular and short carboxyterminal cytoplasmic domains connected by a single transmembrane segment¹ (Fig 1, *bottom*). The extracellular domain of many, but not all, integrins binds to an Arg-Gly-Asp-Ser (RGDS) adhesive sequence contained in many extracellular matrix components such as fibronectin, thrombospondin, fibrinogen and vitronectin.¹⁷ Ligand binding is dependent on divalent cations as well as surrounding sequences within the ligand. Intracellularly, integrins are believed to interact with the actin cytoskeleton through α -actinin, vinculin and talin providing a link between the extracellular space and the intracellular compartment.²⁰

In general, fibronectin receptors are classified within the β_1 subfamily of integrins (also termed VLAs). The VLA 5 ($\alpha_5\beta_1$) is known as the classic fibronectin receptor since it was isolated first and mediates many of the cellular effects of fibronectin including cell adhesion and migration. The VLA-5 also is important for fibronectin matrix assembly.^{21,22} Other fibronectin receptors within the β_1 subfamily include VLA-3 ($\alpha_3\beta_1$), which also binds to laminin and collagen,

and VLA-4 ($\alpha_4\beta_1$). Both VLA-5 and VLA-3 bind to the RGD sequence present in the mid portion of the fibronectin molecule. The VLA-4 binds to a variable splicing site found in the carboxyterminal end of fibronectin and is found in leukocytes and certain cancer cells. Interestingly, VLA-4 also serves as a homing receptor for lymphocytes in Peyer's patches.²³

Cell-fibronectin binding also is mediated via surface components other than integrins. For example, proteoglycans also may bind fibronectin.¹ Soluble chondroitin sulfate proteoglycans inhibit amphibian neural crest locomotion on fibronectin. Gangliosides also have been implicated in binding fibronectin. However, most observers believe integrins primarily are responsible for cell adhesion to fibronectin, with proteoglycans and gangliosides modulating their binding. The aminoterminal region of fibronectin contains a 29 kDa fragment which binds to cells and is critical for fibronectin matrix assembly in cultured lung fibroblasts.² The identity of the elusive so-called matrix assembly receptor responsible for this interaction is not known although it could be an integrin-like molecule.²⁴

BIOLOGICAL ACTIONS OF FIBRONECTIN

The initial interaction of cells with immobilized fibronectin is the binding of cell surface fibronectin receptors to adhesive sequences contained within the fibronectin molecule. This binding event activates multiple intracellular processes responsible for cell adhesion and subsequent cell spreading. Many of these intracellular activities are mediated via the integrin VLA-5. Following binding to fibronectin, VLA-5 receptors aggregate or cluster into membrane structures termed adhesive plaques which contain cytoskeletal proteins.²² This is followed by reorganization of the actin cytoskeleton resulting in cell spreading. These events are blocked by antibodies to VLA-5 or reagents that inhibit ligand binding, such as synthetic peptides containing the sequence RGDS. Although VLA-3 also binds to fibronectin, its function *in vivo* is poorly understood. Other membrane components are likely to modulate cell adhesion to fibronectin, and moreover, different cell types (hematopoietic and neural crest cells) may bind to fibronectin using alternative fibronectin receptors.

Although fibronectin typically is known as an adhesive substrate, it also promotes the migration of certain cells including fibroblasts in fibrin clots, avian neural crest cells and other embryonic cells during heart development.²⁵ Both the RGDS site and the IIICS domain of fibronectin support the migration of neural crest cells. It is difficult to envision how an adhesive substrate can promote cell migration. Migration requires the tight regulation of repetitive cell attachment and detachment from a substrate. It is

possible that the binding properties of the fibronectin receptor, and therefore its ability to anchor the cell to the substrate, may be modulated by changes in its conformational state resulting from interaction with extracellular, membrane or intracellular components. In such a manner, receptors may alternate between adhesive and nonadhesive states, thereby permitting directional cell migration.

The proliferation and differentiation of many cell types is dependent on anchorage to a substrate. The ability of fibronectin to promote the proliferation of human lung fibroblasts²⁶ and other cells may therefore be explained by its cell adhesive properties. Of interest is the observation that some hematopoietic cell precursors lose their ability to bind to fibronectin during differentiation and maturation, permitting them to egress from the bone marrow.²⁷ Another example of fibronectin's role of differentiation is seen in muscle development, as an antibody that blocks binding to the fibronectin/laminin receptor inhibits myoblast differentiation in the chicken.²⁸

The mechanisms of cellular response to fibronectin are largely unknown. Fibroblast adhesion to fibronectin in tissue culture nearly always is followed by cell shape changes. Previously, it has been hypothesized that many of the cellular effects of fibronectin, including altered gene expression, in part are mediated by cellular shape change and accompanying changes of the cytoskeleton.²⁹ Recently, specific mechanisms of fibronectin signal transduction occurring in the absence of cellular shape change have been reported. For example, fibronectin binding to VLA-5 receptors induces collagenase and stromelysin gene expression in fetal lung fibroblasts, an effect noted in the absence of cell shape change.²⁹ Transmembrane calcium fluxes, receptor and cytoskeletal protein phosphorylation and intracellular pH changes all have been implicated in signal transduction via integrins; however, their role in fibronectin receptor function has not yet been determined.

FIBRONECTIN IN THORACIC DEVELOPMENT

Human embryogenesis is characterized by remarkable morphologic changes resulting in the formation of specialized tissues. Intricate regulation of biologic events such as cell adhesion, migration, cytodifferentiation and proliferation is essential for the normal progression of organogenesis. As described earlier, fibronectin is highly expressed in embryonic tissues and has been shown to affect many of the cellular processes required for embryogenesis *in vitro*.^{4,25,30} It is therefore not surprising that a functional role for fibronectin has been suggested in both cardiac and pulmonary development.³¹⁻³³ However, due to the complex nature of the extracellular matrix, it has been difficult to ascertain the exact effects of fibronectin

and other matrix components on specific cellular events during embryogenesis *in vivo*. Most of our knowledge of fibronectin's function during development is derived from studying its effects on cultured embryonic cells and its spatial and temporal distribution in developing tissues.

Development of the lung is divided into four stages: the embryonic, glandular, vascular and alveolar stages.³⁴ The human lung derives from an epithelial outpouching of the epithelial foregut during the embryonic period (1 to 7 weeks). Subsequently, the primordial airways are formed through a process termed branching morphogenesis during the glandular stage (Fig 2) (5 to 7 weeks). Branching morphogenesis is characterized by repetitive monochotomous and dichotomous branching in the presence of continuing cell proliferation forming multiple tubules surrounded by mesenchyme. This primitive bronchial tree is invaded by vessels during the vascular stage (16 to 26 weeks) and serves as the frame for subsequent formation of gas-exchange units during the alveolar stage of lung development (27 weeks to age 8 postnatally). High expression of fibronectin is typical of developing lungs, as noted by Chen et al,³³ and J. Roman, M.D., and J. A. McDonald, M.D. (unpublished data). This is particularly true during the glandular stage, where its expression coincides with the period of maximal cell proliferation.

In early glandular stage mouse lungs, fibronectin is present at the epithelial-mesenchymal interface in developing airways and within the surrounding mesenchyme. During the mid-glandular stage, fibronectin expression increases predominantly at the epithelial-mesenchymal interface and around mesenchymal smooth muscle cells adjacent to the developing airways (parabronchial smooth muscle cells), as noted by Heine et al³⁵ and by J. Roman, M.D. and J. A. McDonald, M.D. (unpublished data). During the vascular stage, fibronectin also is expressed in vessels. Although the expression of fibronectin appears to decrease afterwards, recent investigations have documented increased fibronectin content in newborn rat lungs, suggesting a role for fibronectin in alveolar formation during the early postnatal period as well.³⁶

During the glandular stage, fibronectin is concentrated at regions of cleft formation, demarcating future airway bifurcations where it is codistributed with glycosaminoglycans, collagens, laminin and TGF- β .³⁵ These findings suggest a central role for fibronectin and other extracellular matrix components in lung branching morphogenesis. Reagents that block fibronectin matrix assembly in cultured lung fibroblasts are capable of inhibiting murine lung branching *in vitro*.³⁷ This finding provides further evidence for the role of fibronectin matrices in branching of the developing airways.

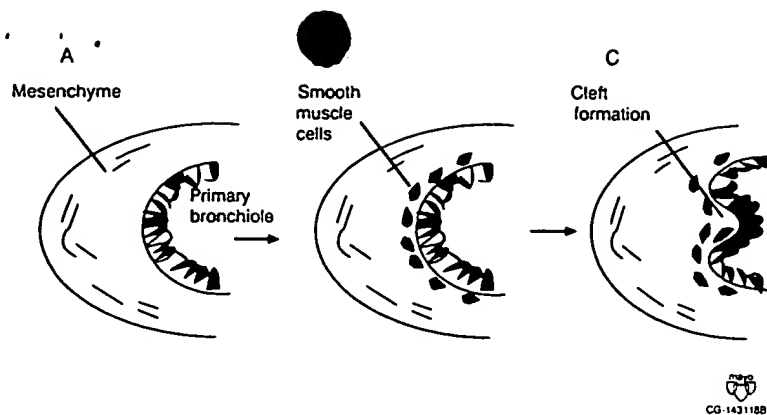


FIGURE 2. Model of branching morphogenesis in the developing lung. A: During the early glandular stage, the lung contains few branches surrounded by mesenchyme-containing fibronectin. B: Subsequently, fibronectin may promote the migration or differentiation of parabronchial mesenchymal cells into smooth muscle. C: These parabronchial smooth muscle cells alter the composition of the extracellular matrix promoting cleft formation and airway bifurcation.

Early glandular stage lungs also express fibronectin receptors VLA-3 and VLA-5.³⁹ The VLA-3 is detected most prominently in airway epithelial cells perhaps assisting in cell-cell interactions, while VLA-5 receptors appear to codistribute with fibronectin in parabronchial smooth muscle cells and within the mesenchyme. The role of these and other integrin receptors in lung development has not been fully established; however, RGD-binding integrin receptors seem important as RGD peptides inhibit normal lung branching morphogenesis in murine lungs grown as explants in tissue culture.³⁸

The tight correlation between the expression of fibronectin, laminin, collagen type 1 and the integrin receptor VLA-5 in embryonic parabronchial smooth muscle cells suggest a potential role of these cells during lung development. We hypothesize that parabronchial smooth muscle cells migrate or differentiate around developing airways at specific sites of cleft formation and transform the composition of the extracellular matrix into one suitable for branching (Fig 2). This is supported by our observation that heparin, a glycosaminoglycan that has tremendous effects on smooth muscle migration and proliferation, blocks murine branching morphogenesis *in vitro* (unpublished observations). Fibronectin may help direct the migration of these parabronchial smooth muscles around developing airways as well as control cell differentiation at sites of cleft formation. We have additionally noted that fibronectin deposition occurs specifically at the region of cleft formation and not at the growing tips of the branching airways. The absence of fibronectin at the tips may allow further growth, whereas its presence at the cleft may prevent outgrowth of tissue and promote directional branching.

Fibronectin also is likely to be involved in several stages of cardiac development. Heart development has been divided in three stages: early (2 to 4 weeks), advanced (4 to 8 weeks) and fetal (8 weeks to birth). During the early phase, precardiac cells present in the lateral mesodermal zones of the embryo migrate anteriorly toward the midline where they form the

tubular heart. The appearance of fibronectin at the mesoderm-endoderm interface coincides temporally and spatially with the migration of these precardiac cells.³² Anti-fibronectin antibodies or the disruption of these fibronectin-containing migratory pathways prevent normal heart development in the chicken, which supports the idea that fibronectin matrices play a critical role in the formation of the early tubular heart.³¹

Fibronectin also may be important during the second or advanced stage of cardiac development. During this phase, the heart is septated into multiple chambers and the cardiac valves are formed via a process termed epithelial-mesenchymal transformation. Epithelial-mesenchymal transformation is characterized by the migration of endothelial cells into the cardiac jelly or endocardial cushions (the cell-free extracellular matrix located between the endothelium and the myocardium). Once within the cardiac jelly, the endothelial cells transform into mesenchyme. Fibronectin expression is increased at the outer layer of the heart during this stage, suggesting that fibronectin may promote the migration of VLA-5-armed endothelial cells into the cardiac jelly (unpublished data, J. Roman, M.D., and J. A. McDonald, M.D.). Formal proof for this hypothesis, however, is currently lacking.

FIBRONECTIN IN LUNG REPAIR AND FIBROSIS

Acute lung injury is characterized by interstitial edema, formation of alveolar exudates, disruption of basement membranes and tissue invasion by inflammatory cells.³⁹ In general, regardless of the causative agent, the lung responds in a stereotypical manner. Pulmonary injury caused by such diverse agents as smoke, acid, drugs or multiorgan disease results in a generalized activation of counterreactive mechanisms responsible for ridding the lung of the injurious agents and effecting tissue repair. Following the initial insult, the affected area is infiltrated by inflammatory cells, predominantly neutrophils. The influx of inflammatory cells largely is mediated by cytokines such as leuko-

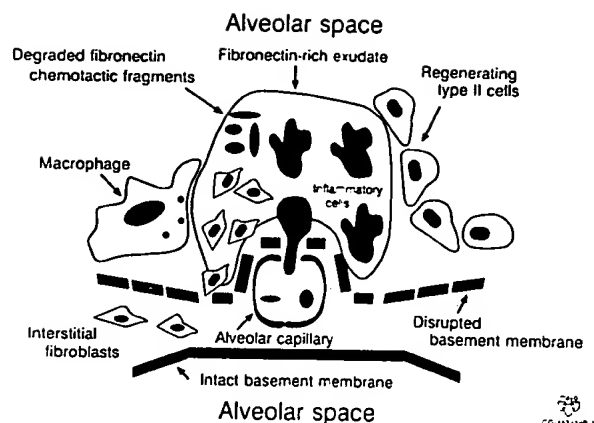


FIGURE 3. Potential roles for fibronectin in lung repair and fibrosis. Following injury to the basement membrane the alveolar space becomes flooded with plasma containing fibronectin. This fibronectin-rich exudate promotes the migration and proliferation of inflammatory cells, fibroblasts and epithelial cells. Organization of the exudate by fibroblasts leads to the deposition of cellular fibronectin, collagen and proteoglycans. Exuberant extracellular matrix deposition and reepithelialization of the organized exudate results in pulmonary fibrosis.

triene B₄, interleukin-1, tumor necrosis factor- α and γ -interferon derived from alveolar macrophages responding to the injury.⁹ These cytokines may facilitate the attachment of leukocytes to the endothelium of blood vessels by stimulating the expression of adhesion molecules on vascular endothelial cells.¹⁰ Once adherent to endothelium, leukocytes invade the lung parenchyma and migrate to the affected area where they release mediators responsible for the recruitment of mononuclear cells and fibroblasts.

The inflammatory phase is followed by a second or reparative phase in which cells invade the tissue. Activated monocytes release reactive oxygen species, proteases capable of degrading extracellular matrix components and cytokine growth factors. Cytokine growth factors stimulate the recruitment of fibroblasts and other cell types, enhance new matrix deposition and regulate neovascularization and reepithelialization of injured tissues. Subsequently, tissue repair is accomplished by extracellular matrix deposition and tissue remodeling. During this phase, abnormal or uncontrolled reparative mechanisms may result in fibrosis. The ability of the normal reparative mechanisms to maintain the original architecture of the lung depends on a number of variables including host inflammatory responses, the nature of the injurious agents, the time of exposure, and most notably, whether the injury has destroyed the continuity of the basement membranes which maintain the normal lung architecture.³⁹

Pulmonary injuries resulting in abnormal repair and scarring have been termed the fibroproliferative lung disorders and include AIP, the organizing phase of ARDS, BOOP, drug-induced pulmonary fibrosis, inorganic dust pneumoconioses and IPF. Lung biopsies

from patients with fibroproliferative diseases possess substantially increased numbers of fibroblasts and an altered fibroblast phenotype associated with the synthesis of multiple extracellular matrix components including fibronectin, proteoglycans and collagens, types 1 and 3. Therefore, in a manner reminiscent of the developing lung, pulmonary repair and fibrosis are characterized by a generalized stimulation of matrix gene expression and deposition of new matrix proteins.

Several distinctive histologic patterns occur in patients with fibroproliferative lung disease. The most common pattern of fibrosis results from organization of alveolar exudates (Fig 3). Disruption of the alveolar-capillary barrier results in flooding of the alveoli. The resulting alveolar exudate is invaded by newly formed buds of inflammatory and mesenchymal cells that penetrate the disrupted basal lamina in regions of epithelial injury and denudation. Infiltrating fibroblastic cells secrete fibronectin and procollagen type 1. Proliferating type 2 cells migrate over the organizing exudate, incorporating it into a markedly thickened alveolar septum. This pattern of fibrosis has been observed in a majority of patients with ARDS and IPF.⁶ Less commonly, fibrosis occurs by local proliferation of fibroblasts with accompanying secretion of matrix proteins resulting in a direct expansion of the alveolar interstitium.⁶ This pattern may accompany intraalveolar fibrosis in IPF, but is usually observed in patients with acute pulmonary fibrosis such as that seen with AIP. In other regions of lung injury, denuded alveoli may collapse upon themselves. The basement membranes of opposite sides of the alveoli are fused and folded together.³⁹

Under normal conditions, adult lungs contain plasma fibronectin in basal lamina of alveolar capillaries and epithelium, in basal lamina of conducting airways, in pleural and alveolar lining fluid and around smooth muscle cells in the interstitial spaces.⁴¹ In contrast, very little cellular fibronectin is present in healthy pulmonary tissue. However, after lung injury, a tremendous increase in fibronectin expression is detected in basal lamina and in the lung parenchyma.⁴¹ Abundant fibronectin has been noted in lung parenchyma and intraalveolar exudates of patients with ARDS as well as in more chronic examples of lung injury such as IPF and sarcoidosis.^{42,43} Similarly, increased fibronectin mRNA has been detected in animal models of acute lung injury including bleomycin as well as in the epithelial lining fluid of animals exposed to inhaled heavy metals, paraquat and radiation.²⁵

During acute lung injury, the interstitial and alveolar spaces are flooded with plasma. Therefore, much of the fibronectin detected in injured lungs is of plasma origin. However, fibronectin also is locally synthesized by activated alveolar macrophages, fibroblasts, endo-

thelial cells and alveolar type 2 and bronchial epithelial cells in injured pulmonary tissues. Local production of fibronectin has been documented in lung biopsies of patients with fibroproliferative lung disorder.⁶ Studies employing immunohistochemistry with monoclonal antibodies specifically recognizing cellular fibronectin and *in situ* hybridization localization of fibronectin mRNA demonstrate increased fibronectin production by fibroblasts and alveolar macrophages in IPF, AIP, BOOP and the organizing phase of ARDS.^{6,7,44}

The enhanced expression of fibronectin during lung injury is likely to be regulated by a complex network of cytokines, most notably TGF- β . The TGF- β , a cytokine derived from platelets and activated alveolar macrophages, causes an overall stimulation of gene expression for fibronectin, the VLA-5 fibronectin receptor, collagen and other components of the extracellular matrix *in vitro*.^{17,45} Recently, TGF- β has been implicated in the fibroproliferative repair process observed in patients with IPF. Lung biopsies demonstrate a striking colocalization between regions of TGF- β deposition and associated matrix gene activation in patients with IPF.⁴⁴ The TGF- β is secreted and concentrated in regions of pulmonary fibrosis where it may further stimulate extracellular matrix deposition.

The roles of fibronectin in lung injury and fibrosis are poorly understood. Interestingly, the splicing pattern of fibronectin at sites of injury closely resembles that derived from embryonic tissues, suggesting that specific forms of fibronectin may be critical for events necessary for wound repair.⁴⁶ During lung injury, fibronectin fragments resulting from increased proteolytic activity are chemotactic to monocytes, promoting influx of inflammatory cells into regions of lung damage (Fig 3).⁴⁷ Fibronectin deposited within the airspaces also may promote the migration of fibroblasts into regions of alveolar damage.⁴⁸ It also has been postulated that fibronectin may serve to organize the subsequent deposition of collagen in the extracellular matrix. Antibodies to the collagen binding region of fibronectin have been noted to inhibit the subsequent deposition of both fibronectin and collagen matrix by lung fibroblasts *in vitro*.⁴⁹

FIBRONECTIN IN CARDIOPULMONARY INFECTION

Adherence of microorganisms to host epithelial surfaces is necessary for the establishment of infection in the skin, urinary system and upper and lower respiratory tracts. Microorganisms have evolved widely varied mechanisms to attach to host epithelial cells including specialized structures such as pili and fimbriae. Recently, it has been found that some microbes also may exploit host adhesive proteins, such as fibronectin, to attach to epithelial surfaces. Follow-

ing adhesion and local colonization of epithelial surfaces, invasive organisms penetrate, destroy adjacent host tissues and spread from the region of entry. Less virulent organisms may remain attached to the host epithelial surface where they may inflict damage locally or secrete toxins with regional and systemic effects. The proliferation of pathogenic microorganisms is principally combatted by phagocytic cells such as the neutrophil and macrophage which are aided by components of the humoral and cellular immune systems.

Fibronectins interact with a large number of microorganisms including bacteria, yeasts and protozoans (Table 1). The mechanisms of host-parasite interaction and the biological impact of these interactions are profoundly different in the various species studied. In 1978, Kuusela⁵⁰ reported that plasma fibronectin binds to *Staphylococcus aureus*. The binding site of *S aureus* has been mapped to the aminoterminal of fibronectin, and subsequent work has identified a 200 kDa surface protein of *Staphylococcus* which is the receptor interacting with this portion of the molecule.⁵¹ The interaction of fibronectin with staphylococci in most cases appears to benefit the bacterium, as the number of fibronectin receptors and the ability of fibronectin to agglutinate bacterial isolates has been found to correlate with the invasiveness of various strains of *S aureus*.⁵² The ability of staphylococci to adhere to fibronectin also permits the organisms to colonize blood clots, regions of denuded vascular endothelium and serum-coated materials in the vascular space such as indwelling catheters, cardiac valves or vascular

Table 1—Interaction of Fibronectin with Microorganisms

Organism	Mechanism of Binding	Significance of Fn Binding
<i>S aureus</i>	200 kDa surface protein	Binding to endothelial cells and phagocytes
<i>Strep pyogenes</i>	Lipoteichoic acid; surface protein	Oral colonization
<i>E coli</i>	Some strains bind via curlin (17 kDa)	Epithelial adherence
<i>Treponema pallidum</i>	89 kDa protein which recognizes RGD	Correlates with virulence
<i>Candida albicans</i>	Possibly a β_1 integrin-like molecule	Binding to epithelial cells
<i>Trypanosoma cruzi</i>	Putative 85 kDa receptor	Binding to macrophages
<i>Leishmania chagasi</i>	Undefined	Binding to monocytes
<i>Entamoeba histolytica</i>	Undefined	Protease release by parasite
<i>P carinii</i>	Surface glycoprotein; gp120	Adherence to cultured lung cells

grafts. Fibronectin-augmented adherence may thereby permit the organism to gain a foothold in such intravascular infections as catheter-associated sepsis and bacterial endocarditis. Whether fibronectin plays a role in staphylococcal pneumonia is currently unknown. Fibronectin coating of staphylococci increases the binding of organisms to macrophages and neutrophils but does not appear to augment their uptake.⁵³

Other Gram-positive organisms such as streptococcal species also bind fibronectin and use this adhesive protein as a potential means of attachment to oral and oropharyngeal epithelial cells. The mouth, teeth and oropharynx are covered with fibronectin derived from the salivary secretions. As in the case of *S aureus*, fibronectin interacts with *Streptococcus pyogenes* through its aminoterminal portion.⁵⁴ Fibronectin appears to interact both with lipoteichoic acid and a protein present in the bacterial cell wall.^{55,56} The binding of pathogenic streptococci to the teeth and the oral mucosa is an important component of normal oral colonization and is of potential importance in dental infection and infections of the upper respiratory epithelium.

The interaction of Gram-negative bacteria with fibronectin is more complex than that noted for Gram-positive organisms. Pathogenic Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marsecens* exhibit limited binding to fibronectin.^{57,58} Fibronectin coating of buccal cells favors attachment of less pathogenic Gram-positive organisms such as streptococci and limits the binding of virulent Gram-negative organisms.⁵⁹ The oropharyngeal secretions of critically ill patients possess enhanced proteolytic activity which may act to degrade this normal fibronectin coating resulting in enhanced adherence of pathogenic Gram-negative organisms to epithelial cells of the oropharynx.⁶⁰⁻⁶² Oropharyngeal attachment of Gram-negative bacteria such as *P aeruginosa* precedes the subsequent colonization of lower airways and nosocomial pneumonia in critically ill patients.

Recent investigations also indicate that fibronectin may be important in the pathogenesis of *Pneumocystis carinii* pneumonia.^{63,64} *Pneumocystis carinii* is predominantly an intraalveolar parasite which causes severe and often fatal pneumonia in immunocompromised patients including those with AIDS. The adherence of *P carinii* to the alveolar epithelium is a central component of the parasitic life cycle and in the initiation of pneumonia. The mechanisms by which *P carinii* adhere to alveolar epithelial cells are currently the focus of intense study. *Pneumocystis carinii* attaches to cultured epithelioid lung cells in a manner similar to that occurring in human tissues as visualized by electron microscopy.^{65,66} *Pneumocystis carinii* has been demonstrated to bind fibronectin through ap-

proximately 6.4×10^5 binding sites per organism.⁶³ Addition of antifibronectin antibodies results in reduced *P carinii* adherence to cultured lung cells, an effect which is reversible by the addition of exogenous fibronectin.⁶³ It has been further reported that fibronectin interacts with a specific glycoprotein on the parasite termed gp120.⁶⁷ In view of the local abundance of fibronectin in alveolar lining fluid, *P carinii* binding to fibronectin might assist attachment to the alveolar epithelial cell *in vivo*.

It has been suggested that coating of microorganisms with fibronectin might potentiate their uptake by phagocytic cells equipped with appropriate fibronectin receptors. This process of facilitated phagocytosis has been termed nonimmune opsonization. The notion that fibronectin might act as such an opsonin has been strengthened by the observation that fibronectin-coated polystyrene beads not only are bound to cultured fibroblasts but also are internalized by these cells.⁶⁸ However, the evidence that fibronectin acts as a direct opsonin for phagocytic cells such as the alveolar macrophage is not conclusive. Although monocytes and macrophages clearly bind fibronectin and fibronectin has been reported to enhance the phagocytosis of gelatin-coated erythrocytes or lipid particles, fibronectin alone is not effective in promoting uptake of particles by phagocytes.^{69,70} In each case described, fibronectin acts to potentiate the interaction of some other ligand with the phagocytic cell. Therefore, fibronectin should be viewed as a potentiator of phagocytosis rather than as a direct opsonin.

CURRENT AND FUTURE RESEARCH

Although much has been learned of fibronectin's structure, function and potential role in disease, several important topics provide the basis for ongoing investigation. The control of fibronectin expression continues to be an area of active research. Recent investigations have led to cloning of the fibronectin gene and promoter.^{71,72} Study of these sequences will provide further insight into the control of fibronectin gene expression and may eventually lead to novel therapeutic approaches to ameliorate the increased fibronectin expression observed in pulmonary fibrosis.

The role of fibronectin in thoracic development also is being further defined. Developmental effects of many proteins are best observed in patients or animals deficient in the molecule. A naturally occurring fibronectin deficiency state has not been documented, perhaps reflecting fibronectin's central role in embryogenesis. Currently, transgenic animals with deficient and mutated fibronectins are being produced. These animals should provide useful model systems to determine the exact role of fibronectin in cardiopulmonary development and may yield further insight into whether fibronectin plays a role in congenital abnor-

malities of the heart and lung.

Finally, other investigations are under way to determine the mechanisms by which soluble fibronectin is assembled into an insoluble matrix. Exuberant deposition of fibronectin-rich matrices is an early event in lung repair and fibrosis. Fibronectin matrix assembly requires a complex interaction of two regions of the fibronectin molecule with two separate receptors on matrix-forming cells. The first domain is fibronectin's Arg-Gly-Asp (RGD) cell adhesive sequence which binds to VLA-5 integrin receptors and the second region is contained within fibronectin's aminoterminal region which interacts with another uncharacterized cell surface receptor.² Inhibition of either region of fibronectin from binding with its corresponding receptor results in partial inhibition of fibronectin matrix assembly.² Better understanding of the mechanisms of fibronectin matrix deposition and the development of agents which interrupt this process may provide novel therapies for the fibroproliferative repair process observed in fibrotic lung disease.

SUMMARY

Fibronectin, a dimeric glycoprotein, utilizes its multidomained structure to interact with components of the extracellular matrix, microorganisms and mesenchymal, epithelial and inflammatory cells. Fibronectin interacts with these cells through specific cell surface receptors including members of the integrin family which mediate many of fibronectin's effects on cellular function such as promotion of cell adhesion, proliferation, migration and cytodifferentiation. These varied biologic activities account for fibronectin's central involvement in embryogenesis, repair of injured tissues, tissue fibrosis and infection. Recent investigations document important functions for fibronectin in normal cardiopulmonary development as well as in the repair of lung injury and pulmonary fibrosis. Due to its interaction with microorganisms, fibronectin also is potentially important in the adherence of microbes to epithelial cells and in the establishment of cardiac and respiratory infections. Additional exciting investigations will more fully define the control of fibronectin gene expression, the mechanisms of fibronectin matrix assembly and the potential roles for fibronectin in cardiopulmonary disease.

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